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A Practical Synthesis of Multitargeted Antifolate LY231514

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Abstract:

A concise and scalable synthesis of LY231514 (1), a new pyrrolo-[2,3-*d*]pyrimidine-based antitumor agent, is presented. Reaction of 2-bromo-4-arylbutanal 9 with 2,4-diamino-6-hydroxypyrimidine (10) regioselectively provided pyrrolo[2,3-*d*]pyrimidine 11, representing the core structure of the drug, in good yield. Assimilation of the glutamic acid residue by conventional means completed the synthesis. Development of the optimized synthetic route emphasized avoiding isolation of the relatively unstable aldehyde and bromoaldehyde intermediates.

Introduction

The concept that inhibitors of folate-requiring enzymes could arrest the proliferation of malignant cells is perhaps one of the oldest paradigms of cancer chemotherapy and resulted in the discovery of methotrexate, one of the first mechanism-based cancer drugs, more than 50 years ago.[1] Since that time, substantial research efforts have led to better understanding of folate biochemistry and the mechanism of methotrexate cytotoxicity, thus stimulating efforts to prepare inhibitors of folate-requiring enzymes with improved clinical utility in the treatment of cancer. The pyrrolo[2,3-*d*]pyrimidine-based folate analogue, LY231514 (**1**, Figure 1), first prepared by Taylor and co-workers at Princeton University and evaluated for antitumor activity at Lilly, represents a new generation of folate-requiring enzyme inhibitors with improved antitumor potency and spectrum.[2] LY231514 has recently been found to possess inhibitory activity against dihydrofolate reductase and C-1 synthase in addition to its primary enzymatic target, thymidylate synthase, and has thus been designated as a multitargeted antifolate (MTA).[3] Phase II clinical trials have revealed clinically significant activity against colorectal, lung, and breast cancer and promising activity against a number of other tumor types. We describe herein the development of a practical synthesis of LY231514 which is more suitable for support of advanced drug development efforts.[4]

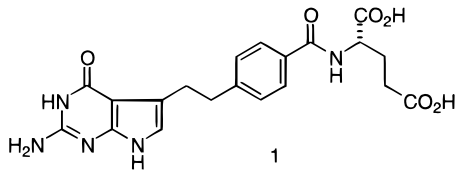

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Figure 1.

Taylor's initial synthesis of LY231514 focused on the construction of the two-carbon linker between the pyrrolopyrimidine and aryl glutamate moieties.[2] Sequential palladium(0) coupling of acetylene (initially protected as the TMS derivative) to aromatic (**2**) and heterocyclic (**4**) halides, corresponding to the desired fragments as indicated in Scheme 1, provided (via **3**) unsymmetrical acetylene intermediate **5**. Conversion of **5** to **1** by standard methods of catalytic reduction and hydrolytic removal of the ester and amine protecting groups completed the synthesis. This conceptually elegant and useful synthesis of this series of antitumor agents lent itself readily to the preparation of **1** and its analogues for SAR studies.

This flexibility came at the price, however, of requiring four steps to construct the ethylidene linker (two palladium-mediated acetylene coupling operations, desilylation, and catalytic reduction), in addition to the synthesis of the aryl and heterocyclic coupling partners.[5] The required 5-iodopyrrolo[2,3-*d*]pyrimidine intermediate proved difficult to prepare. Direct iodination of 2-pivaloylamino-4-hydroxypyrimidine with *N*-iodosuccinimide had given mixtures of 5- and 6-iodinated products as well as the 5,6-diiodide and starting material in the Taylor laboratory,[2] and our experience with this reaction was the same. For good results, it was necessary to deliberately 5,6-diiodinate the pyrrolopyrimidine intermediate and remove the 6-iodo substituent by selective reduction with zinc in acetic acid. The zinc reduction required careful control of stoichiometry and conditions to avoid under- or overreduction (deiodination of the 5-position). These requirements proved difficult to provide as the scale increased. We developed an improved procedure for the preparation of **4** involving in situ N,O-bis-silylation of the pyrrolopyrimidine precursor and regiospecific C-5 monoio-

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(1) For recent overviews of antifolate research related to antitumor therapy, see: Berman E. M.; Werbel, L. M. *J. Med. Chem.* **1991**, *34*, 479 and references therein. Taylor, E. C. *J. Heterocycl. Chem.* **1990**, *27*, 1−12 and references therein.

(2) Taylor, E. C.; Kuhnt, D.; Shih, C.; Rinzel S. M.; Grindey, G. B.; Barredo, J.; Jannatipour, M.; Moran, R. G. *J. Med. Chem.* **1992**, *35*, 4450−4454.

(3) Shih, C.; Chen, V. J.; Gossett, L. S.; Gates, S. B.; MacKellar, W. C.; Habeck, L. L.; Shackelford, K. A.; Mendelsohn, L. G.; Soose, D. J.; Patel, V. F.; Andis, S. L.; Bewley, J. R.; Rayl, E. A.; Moroson, B. A.; Beardsley, G. P.; Kohler, W.; Ratnam, M.; Schultz, R. M. *Cancer Res.* **1997**, *57*, 1116−1123.

(4) A preliminary report of this work has been published: Barnett, C. J.; Wilson, T. M.; Kobierski, M. E. In *Chemistry and Biology of Pteridines and Folates 1997*; Pfleiderer, W., Rokos, H., Eds.; Blackwell Wissenschafts-Verlag: Berlin, 1997; pp 123−126.

(5) Intermediate **2** could be prepared from 4-iodobenzoyl chloride and dimethyl-L-glutamate but is not commercially available. Preparation of **4** proceeded via (commercially unavailable) 2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidine (acylation with pivaloyl chloride, iodination, and zinc reduction as described by Taylor et al.[2]). The required pyrrolo[2,3-*d*]pyrimidine starting material could be prepared by literature methods from, for example, 2,4-diamino-6-hydroxypyrimidine, as described in ref 7.

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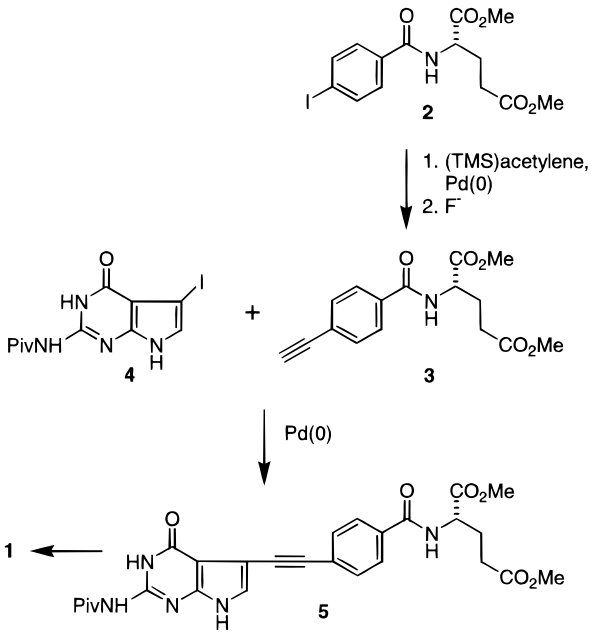
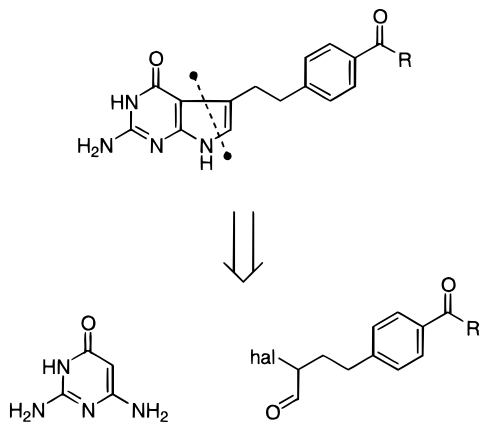
Scheme 1



dination of the resulting silylated intermediate.[6] The latter method represented a significant improvement, but the experimental challenges and expense of the two palladium-catalyzed coupling steps remained unremedied. The coupling of acetylenic intermediates to the iodinated pyrrolopyrimidine system was complicated by a strong tendency for the iodoheterocycle to undergo reductive deiodination rather than coupling, causing the yield of desired product to be moderated.

Synthetic Strategy

We considered that our reliance on palladium coupling chemistry might be significantly reduced by focusing on the formation of the pyrrolo[2,3-*d*]pyrimidine ring system as a point of synthetic convergence, as indicated retrosynthetically below. In particular, the problems involved in preparing the 5-iodopyrrolo[2,3-*d*]pyrimidine **4** and coupling it to the arylacetylene fragment could be circumvented by this approach.



(6) Barnett, C. J.; Kobierski, M. E. *J. Heterocycl. Chem.* **1994**, *31*, 1181−1183.

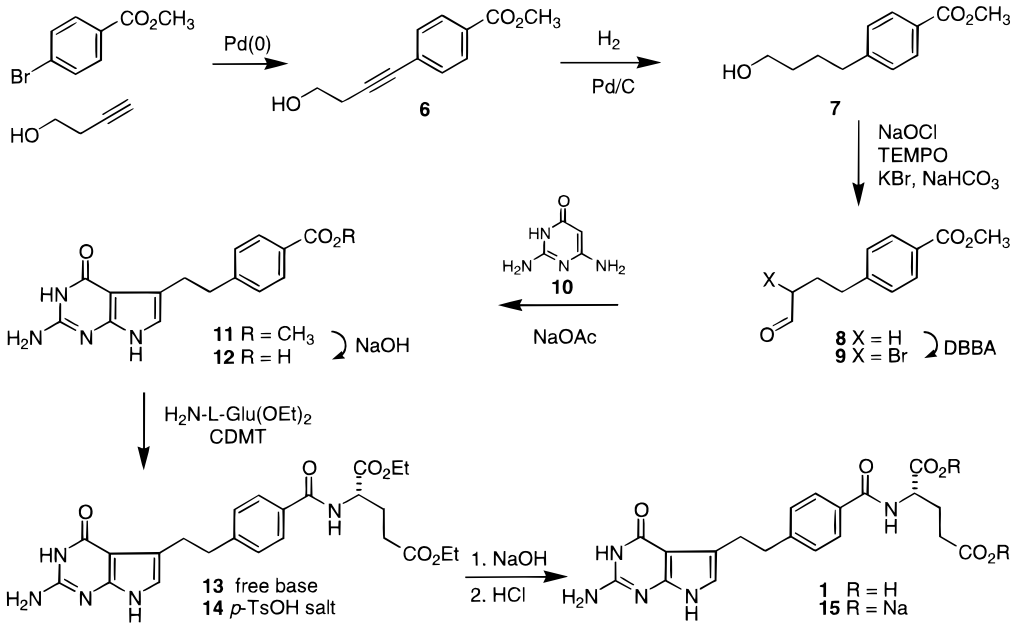
It appears that the first examples of synthesis of pyrrolo-[2,3-*d*]pyrimidines via cyclocondensation of a 2-amino-4-pyrimidone with α-haloaldehydes were carried out by Hitchings and Noell and Robins.[7] These authors reported that the reaction of chloroacetaldehyde with 2,4-diamino-6-hydroxypyrimidine derivatives afforded 2-amino-4-hydroxy-pyrrolo[2,3-*d*]pyrimidines. Secrist and Liu extended the process to the synthesis of various 5- and 6-substituted pyrrolo[2,3-*d*]pyrimidines by utilizing various α-halo ketones and aldehydes.[8] These workers established that fast alkylation of the pyrimidine ring at C-5 by the halocarbonyl compound tended to predominate over attack at other sites (O, N), thus reducing the possibilities for formation of regioisomeric products. Competing alkylation of the pyrimidone oxygen was observed in the case of certain haloketone examples, leading to mixtures containing both pyrrolo- and furo-fused pyrimidine products, but a limited number of haloaldehyde examples were reported to give only pyrrolo[2,3-*d*]pyrimidines. Reaction of 2,4-diamino-6-hydroxypyrimidine with 2-chloropropionaldehyde in dimethyl sulfoxide (DMSO) in the presence of a substoichiometric amount of sodium bicarbonate gave, for example, 2-amino-4-hydroxy-5-methylpyrrolo[2,3-*d*]pyrimidine in 60% (crude) yield. We were encouraged by the reported regioselectivity of the halopropionaldehyde example; the regioisomeric 6-methyl substituted product was apparently not observed. Because of the limited number of examples of this reaction reported here or elsewhere, however, the effect that a more complex haloaldehyde structure would have on the outcome of the reaction was not clear.

Results and Discussion

Our synthetic scheme for utilization of the haloaldehyde cyclocondensation process in the synthesis of LY231514 is depicted in Scheme 2. Palladium(0) coupling of methyl 4-bromobenzoate with 3-butyn-1-ol by a modified literature procedure[9] gave crystalline **6**, which was reduced over palladium on carbon in methylene chloride. Filtration of the catalyst afforded a methylene chloride solution of alcohol **7** (in quantitative yield), which was utilized directly in a TEMPO[10]-catalyzed sodium hypochlorite oxidation[11] procedure, providing the known[12] aldehyde **8**, which was not isolated. Addition of 5,5-dibromobarbituric acid[13] (DBBA) and a small amount of HBr in acetic acid to the methylene chloride solution of **8** effected conversion to the α-bromoaldehyde **9**. Choice of the dibromobarbituric acid reagent was made on the basis of its selectivity toward α-bromination[14] and the convenience of manipulation of a stable, crystalline brominating reagent. Filtration of the reaction mixture removed byproduct barbituric acid,[15] and any remaining brominating agent was removed by washing the solution with

(7) Hitchings, G. H. British Patent 812,366, 1959; *Chem. Abstr.* **1960**, *54*, 592i. Noell, C. W.; Robins, R. K. *J. Heterocycl. Chem.* **1964**, *1*, 34−41.
(8) Secrist, J. A.; Liu, P. S. *J. Org. Chem.* **1978**, *43*, 3937−3941.
(9) Taylor, E. C.; Harrington, P. M. *J. Org. Chem.* **1990**, *55*, 3222−3227.
(10) 2,2,6,6-Tetramethyl-1-piperidinyloxy free radical, Aldrich Chemical Co.
(11) Anelli, P. L.; Montanari, F.; Quici, S. *Org. Synth.* **1990**, *69*, 212−219.
(12) This substance has previously been prepared by the oxidation of **7** with pyridinium chlorochromate (ref 9).
(13) Grundke, G.; Keese, K.; Rimpler, M. *Chem. Ber.* **1985**, *118*, 4288−4291.

Scheme 2



sodium thiosulfate. After aqueous workup, the solution was concentrated and diluted with acetonitrile to effect solvent exchange. Addition of commercially available 2,4-diamino-6-hydroxypyrimidine and aqueous sodium acetate and heating to 45 °C caused cyclocondensation and precipitation of pyrrolo[2,3-*d*]pyrimidine **11** from the reaction mixture in 67% overall yield based on **7**. The yield and quality of the key intermediate **11** from the rather complex sequence beginning with arylbutanol **7** was quite remarkable. Despite numerous formal possibilities for the formation of other bicyclic products, there was no indication of the presence of isomeric cyclization products in the NMR spectra of crude **11**.

The four-step processing sequence for synthesis of **11** (beginning with **6**) was designed to avoid the isolation of oily intermediates **7**, **8**, and **9**, the latter two of which were unstable toward storage conditions as neat liquids.[16] Selection of the conditions for oxidation of alcohol **7** to the corresponding aldehyde **8** proved to be an important consideration. An apparent variant[17] of the Pfitzner−Moffatt oxidation of alcohols involving dimethyl sulfoxide−phosphorus pentoxide in the presence of triethylamine as the oxidant in methylene chloride was successfully utilized. As in all DMSO based oxidation methods, however, removal of the byproduct dimethyl sulfide was difficult unless the aldehyde was isolated and purified by, for example, silica chromatography. When crude **8** was taken to the bromination as a methylene chloride solution (from the workup of the oxidation), a significant amount of dimethyl sulfide was carried into the bromination, consuming the brominating reagent. The dimethyl sulfide could be destroyed by addition of excess brominating agent, but the calculation of the required excess and related analytical work proved to be inconvenient. The TEMPO oxidation provided a solution to this problem by eliminating sulfide residue carryover.

Saponification of **11** with aqueous sodium hydroxide followed by acidification afforded the carboxylic acid derivative **12**, which was elaborated to **13** by means of the chlorodimethoxytriazine active ester[18] peptide coupling method. Reaction of **12** with 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) in the presence of *N*-methylmorpholine in DMF solution followed by reaction of the resulting dimethoxy-*s*-triazinyl ester with diethyl L-glutamate afforded crude **13**, which was isolated via crystallization as the *p*-toluenesulfonate salt (**14**). It is noteworthy that the 2-amino group in **12** did not require protection during the coupling reaction.

Neutralization of the salt and saponification of **13** with aqueous sodium hydroxide followed by acidification with hydrochloric acid gave LY231514 (**1**) as the free diacid, which was crystallized as the disodium salt (**15**) for pharmaceutical use. Careful analytical studies showed that no racemization of the glutamate center occurred in either the coupling or saponification steps.

(14) Oxidation of aldehydes to the corresponding acyl halides is a potential problem in the bromination of aldehydes with elemental bromine, particularly if the reaction mixture is contaminated with water. For a discussion of aldehyde bromination, see: DeKimpe, N.; Verhe, R. In *The Chemistry of* α-*Haloketones,* α-*Haloaldehydes, and* α-*Haloimines*; Patai, S., Rappoport, Z., Eds.; Updates from the Chemistry of Functional Groups; Wiley-Interscience: New York, 1988; Chapter 3. We found *N*-bromosuccinimide and related reagents to be unsuitable for this transformation due to low yields and competing aldehyde oxidation.

(15) The brominating reagent can be conveniently regenerated by bromination of the barbituric acid by bromine. Two moles of Br_2 is required per mole of barbituric acid, but both bromine atoms in the reagent are utilized in the bromination, so the overall stoichiometry is the same as that with bromine itself. See ref 13.

(16) Aldehyde **8** was found to be reasonably stable as an oil at room temperature when chromatographically pure, but the crude material tended to form acetal polymers on standing which were not easily cracked to recover the aldehyde. Bromoaldehyde **9** was unstable toward storage in neat form at refrigerator freezer temperature. It could be kept as a refrigerated (5 °C) methylene chloride solution for 24 h without significant decomposition.

(17) Taber, D. F.; Amedio, J. C.; Jung, K.-Y. *J. Org. Chem.* **1987**, *52*, 5621−5622. The DMSO−P_2O_5−Et_3N method was attractive for large-scale application because the process could be carried out at or near room temperature. The oxalyl chloride-based Swern procedure requires much lower temperatures.

(18) Kaminski, Z. J. *Tetrahedron Lett.* **1985**, *26*, 2901−2904.

The synthesis of LY231514 described herein illustrates the power of retrosynthetic disconnections based on heterocyclic ring synthesis, in this case the pyrrolo[2,3-*d*]pyrimidine system, for providing advantageously convergent and experimentally simple routes to complex synthetic targets. The cyclocondensation of aminopyrimidone **10** with its α-bromoaldehyde partner **9** was the basis for a practical and scalable synthesis of LY231514 (**1**).

Experimental Section[19]

4-(4-Hydroxy-1-butynyl)benzoic Acid Methyl Ester (6). To a mixture of 57.6 kg (268 mol) of methyl 4-bromobenzoate and 420 L of ethyl acetate were added 310 g (1.7 mol) of palladium(II) chloride, 840 g (3.2 mol) of triphenylphosphine, 610 g (3.2 mol) of cuprous iodide, 64 kg (880 mol) of diethylamine, and 23.6 kg (337 mol) of 3-butyn-1-ol. The resulting mixture was heated to 50 °C for about 4 h. The mixture was cooled and filtered, and the filtered solids (diethylamine hydrobromide and residual catalyst) were washed with 180 L of ethyl acetate. The filtrate was washed with a solution of 85 kg of sodium bisulfate in 310 L of deionized water and then separated. The organic layer was washed with saturated sodium chloride. Sodium sulfate (42 kg), silica gel (42 kg), and 18 kg (wet wt) of Deloxan THP[20] resin were added, and the resulting slurry was stirred for about 20 min. The mixture was filtered, and the filter cake was washed with 300 L of ethyl acetate. The filtrate was concentrated under vacuum to 190 L. Ethyl acetate (105 L) was added, followed by 435 L of heptane, causing the product to crystallize. The mixture was cooled to 0−5 °C and stirred for about 2 h. The crystals were filtered, washed with 200 L of cold heptane (0−5 °C), and dried, affording 45.6 kg (83.4% yield) of **6**, mp 97.5−99 °C (lit.[9] mp 95.5−96 °C).

4-(4-Hydroxybutyl)benzoic Acid Methyl Ester (7). A 1.0-kg portion of 5% palladium on carbon catalyst was slurried in 25 L of methylene chloride. The slurry was charged to a nitrogen-purged 30-gal autoclave with an additional 45 L of methylene chloride. Compound **6**, 9.82 kg (48.1 mol), was added with 15 L of methylene chloride. The resulting mixture was stirred under 50 psig of hydrogen for 3.5 h. The catalyst was filtered and washed with 20 L of methylene chloride. The resulting methylene chloride solution was found to contain 9.94 kg of **7** (99.2% based on **6**) by analysis of the mass of the residue from evaporation of an aliquot. The solution of **7** was carried directly into the next step. A small sample was concentrated to dryness in vacuo and identified by NMR spectral comparison with an authentic sample made by the method of Taylor and Harrington.[9]

4-(4-Oxobutyl)benzoic Acid Methyl Ester (8). A solution of 29.0 kg (139 mol) of **7** in 345 L of methylene chloride (prepared in three sections as described above) was reduced to a volume of 155 L by partial distillation of the solvent. To the resulting solution, cooled to −10 to 0 °C, was added 0.045 kg of TEMPO catalyst and a solution of 1.72 kg of potassium bromide in 5.1 L of water. A total of 153 L of 12% aqueous sodium hypochlorite solution (adjusted to pH 9.5 by addition of sodium bicarbonate) was next added at a rate such that the temperature of the reaction mixture did not exceed 20 °C. Complete conversion of starting material was confirmed by HPLC analysis. The layers were separated, and the methylene chloride phase was washed sequentially with a solution of 0.095 kg of potassium iodide in 70 L of 1 N HCl, 70 L of 1 N sodium thiosulfate, 70 L of 5% sodium bicarbonate, and 70 L of half-saturated brine solution. The resulting methylene chloride solution of **8**[21] was taken to the next step without isolation. A sample obtained by concentration of an aliquot from a smaller scale run had ^{1}H NMR data identical to the reported data.[9] The semicarbazone derivative had mp 149−151 °C. Anal. Calcd for $C_{17}H_{13}N_3O_3$: C, 59.30; H, 6.51; N, 15.96. Found: C, 59.25; H, 6.47; N, 15.67.

(*RS*)-4-(3-Bromo-4-oxobutyl)benzoic Acid Methyl Ester (9). The methylene chloride solution of **8** from the previous step was added to a mixture of 24.5 kg (85.8 mol, 171.6 equiv) of 5,5-dibromobarbituric acid[13] (DBBA) and 1.4 L of 30% hydrobromic acid in acetic acid in 150 L of methylene chloride. The mixture was stirred at 25 °C until complete conversion of starting material was indicated by HPLC analysis. The mixture was filtered to remove barbituric acid formed during the bromination. The filtrate was washed with 260 L of 1 N sodium thiosulfate, and the layers were separated. The organic layer was washed with 260 L of 5% sodium bicarbonate solution and 225 L of half-saturated brine solution and then concentrated under vacuum to 90 L. The solution was diluted with 400 L of acetonitrile, and residual methylene chloride was removed from the mixture by distillation, reducing the volume to 255 L. The resulting solution of compound **9**[22] in acetonitrile was taken to the next step without isolation. An analytical sample was prepared separately by flash chromatography. ^{1}H NMR ($CDCl_3$): δ 2.22 (m, 1H), 2.38 (m, 1H), 2.80 (m, 1H), 2.94 (m, 1H), 3.91 (s, 3H), 4.17 (m, 1H), 7.28 (d, J = 8.2 Hz, 2H), 7.98 (d, J = 8.2 Hz, 2H), 9.47 (d, J = 2.2 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 32.6, 32.7, 52.0, 54.3, 128.5, 130.0, 145.2, 166.8, 192.3. IR ($CHCl_3$): 2955, 1720, 1611, 1437, 1284, 1180, 1114. MS (FD): *m*/*z* 286 (100), 284 (98). Anal. Calcd for $C_{12}H_{13}BrO_3$: C, 50.55; H, 4.60. Found: C, 50.31; H, 4.72.

4-[2-(2-Amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoic Acid Methyl Ester (11). To the acetonitrile solution of compound **9** from the preceding step was added 255 kg of deionized water, 16.2 kg (128.6 mol) of 2,4-diamino-6-hydroxypyrimidine (**10**), and 21.1 kg (256.9 mol) of sodium acetate. The slurry was heated to 40 °C for

(19) Proton NMR spectra were obtained at 300 MHz. Carbon-13 NMR spectra were obtained at 75 MHz. NMR peak positions are reported in ppm downfield from internal tetramethylsilane (δ). Other spectral data were obtained using standard instrumentation.

(20) Degussa AG, Frankfurt, Germany. The resin was used to remove residual palladium.

(21) Accelerated rate calorimetry data for **8** as a neat liquid: no exothermic events until slow self-heating (0.04 °C/min) was encountered at 287 °C.

(22) Accelerated rate calorimetry data for **9** as a neat liquid: exothermic decomposition at 85 °C and maximum self-heat rate of 25.5 °C/min at 125 °C. Solution of **9**, 14% in acetonitrile: onset of exotherm at 100 °C with maximum self-heat rate of 0.44 °C at 116 °C. Maximum reaction temperature for conversion of **9** to **11** was limited to 50 °C to provide a 50 °C margin of safety.

about 3 h. The mixture was cooled to 20 °C and filtered. The crystals were washed with 200 L of acetonitrile/water (1:1) and dried under vacuum, affording 29.2 kg (67% yield based on **7**) of **11**, mp > 250 °C. ^{1}H NMR (DMSO-d_6): δ 2.88 (m, 4H), 3.78 (s, 3H), 5.97 (s, 2H), 6.26 (d, J = 1.6 Hz, 1H), 7.29 (d, J = 8.1 Hz, 2H), 7.81 (d, J = 8.2 Hz, 2H), 10.1 (bd s, 1H), 10.6 (bd s, 1 H). ^{13}C NMR (DMSO-d_6): δ 27.4, 35.8, 51.5, 98.3, 113.0, 117.1, 126.6, 128.2, 128.7, 147.9, 150.9, 151.8, 158.9, 165.8. UV (EtOH): λ 225 (ϵ 22 450), 243 nm (ϵ 20 800). Anal. Calcd for $C_{16}H_{15}N_4O_3$: C, 61.53; H, 5.16; N, 17.94. Found: C, 61.42; H, 5.19; N, 17.81.

4-[2-(2-Amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoic Acid (12). Compound **11** (4.76 kg, 15.2 mol) was suspended in 54 L of 2 N aqueous sodium hydroxide solution and heated to 40 °C for about 1.5 h, at which time HPLC analysis showed the reaction to be complete. The reaction mixture was diluted with 81 L of ethanol and cooled to 20−25 °C. The pH was adjusted to 4.4 with 6 N aqueous hydrochloric acid solution, and the resultant precipitate was filtered, washed with 57 L of a 1:1 solution of water and ethanol, and dried to provide 4.12 kg (91%) of compound **12**, mp > 250 °C. ^{1}H NMR (DMSO-d_6): δ 2.86 (m, 4H), 6.11 (s, 2H), 6.28 (s, 1H), 7.26 (d, J = 8.1 Hz, 2H), 7.79 (d, J = 8.1 Hz, 2H), 10.24 (s, 1H), 10.64 (s, 1H), 12.40 (bd s, 1H). ^{13}C NMR (DMSO-d_6): δ 27.3, 35.7, 98.2, 113.0, 117.1, 127.6, 127.9, 128.7, 147.2, 150.2, 151.6, 158.7, 166.8. UV (EtOH): λ 225 nm (ϵ 24 000). Anal. Calcd for $C_{15}H_{14}N_4O_3$: C, 60.40; H, 4.73; N, 18.78. Found: C, 60.59; H, 4.77; N, 18.64.

***N*-[4-[2-(2-Amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic Acid Diethyl Ester (13).** To a suspension of 3.84 kg (12.9 mol) of compound **12** in 22 L of DMF was added 4.04 kg (39.9 mol) of *N*-methylmorpholine, followed by the addition of 3.08 kg (17.5 mol) of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT).[18] The resulting mixture was stirred for 1.5 h at 25 °C, at which time HPLC analysis showed that the reaction was complete. L-Glutamic acid diethyl ester hydrochloride (3.40 kg, 14.2 mol) was added, and stirring was continued at 25 °C until complete conversion of **12** was determined by HPLC. To the reaction mixture were added 71 L of methylene chloride and 71 L of deionized water, and the mixture was stirred for 15 min. The layers were separated, and the lower (organic) layer was concentrated by atmospheric pressure distillation to 30 L and then diluted with 55 L of ethanol. The solution containing **13** was taken directly to the next step. An analytical sample was prepared separately by chromatography on silica gel, mp 169−171 °C. ^{1}H NMR (DMSO-d_6): δ 1.14 (t, 3H), 1.17 (t, 3H), 2.03 (m, 2H), 2.42 (t, J = 7.4 Hz, 3H), 2.89 (m, 4H), 4.02 (q, 2H), 4.08 (q, 2H), 4.41 (m, 1H), 6.00 (s, 2H), 6.29 (d, J = 1.7 Hz, 1H), 7.27 (d, J = 8.2 Hz, 2H), 7.76 (d, J = 8.2 Hz, 2H), 8.82 (d, J = 7.4 Hz, 1H), 10.15 (s, 1H), 10.60 (s, 1H). ^{13}C NMR (DMSO-d_6): δ 14.0, 25.7, 27.9, 30.2, 36.1, 51.9, 59.9, 60.5, 98.7, 113.4, 117.6, 127.3, 128.1, 131.1, 146.6, 151.3, 152.2, 159.3, 166.6, 171.8, 172.2. UV (EtOH): λ 224 nm (ϵ 26 240). Anal. Calcd for $C_{24}H_{29}N_5O_6$: C, 59.62; H, 6.04; N, 14.48. Found: C, 59.40; H, 6.08; N, 14.41.

***N*-[4-[2-(2-Amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic Acid Diethyl Ester 4-Methylbenzenesulfonic Acid Salt (14).** To the resulting solution of **13** was added a solution of 6.12 kg (32.2 mol) of *p*-toluenesulfonic acid monohydrate in 105 L of ethanol, and the resulting suspension was heated under reflux for about 3 h. The mixture was cooled to about 25 °C with stirring. The crystals of **14** were filtered and washed with 85 L of ethanol. The wet cake was reslurried in 155 L of ethanol, refluxed for 1 h, and cooled to 22 °C. The crystals were filtered, washed with 85 L of ethanol, and dried to provide 7.42 kg of **14**. A total of 13.6 kg of **14** thus obtained from separate runs was combined and suspended in 57 L of DMSO. The mixture was heated to 80 °C and stirred for 1.5 h. Hot ethanol (230 L at 75 °C) was added to the solution, which then was cooled to 5−10 °C. The resulting mixture was stirred for 30 min and filtered. The wet cake was reslurried with 175 L of ethanol (5−10 °C), filtered, and washed with 60 L of ethanol. The crystals were dried to provide 11.2 kg (72.4% from **12**) of purified **14**, mp 255−260 °C. Anal. Calcd for $C_{31}H_{37}N_5O_9S$: C, 56.78; H, 5.69; N, 10.68. Found: C, 56.96; H, 5.67; N, 10.79.

***N*-[4-[2-(2-Amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic Acid (LY231514, 1).** Compound **14** (5.5 kg, 8.4 mol) was added to 38 L of 1 N aqueous sodium hydroxide. The solution was stirred under nitrogen at about 25 °C until completion of the reaction was indicated by HPLC analysis. The reaction mixture was diluted with 43 L of ethanol and adjusted to pH 3 with aqueous hydrochloric acid, causing the **1** free acid to precipitate. The resulting slurry was heated with stirring to 70−75 °C and then cooled to 20−25 °C. The crystals were filtered and washed with 50 L of ethanol−water (1:1). The wet cake was reslurried in 54 L of ethanol−water (1:1), filtered, and dried to yield 3.6 kg (100%) of **1**, mp > 250 °C, identical in all respects (NMR, IR, UV, MS, HPLC) with an authentic sample prepared by the method of Taylor et al.[2]

***N*-[4-[2-(2-Amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic Acid Disodium Salt (15).** The disodium salt was obtained by neutralization with sodium hydroxide and crystallization from water−ethanol as a hygroscopic solid in 85% yield. ^{1}H NMR (CD_3-OD): δ 2.25 (m, 4H), 2.95 (m, 4H) 3.28 (dd, J = 1.6, 3.2 Hz, 1H), 6.26 (s, 1H), 7.20 (d, J = 8.2 Hz, 2H), 7.73 (d, J = 8.2 Hz, 2H). Chiral HPLC analysis indicated that the enantiomeric purity of the product was >99.5%.

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